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Histological Structure and Cytomorphometric Characteristics of the Heart of the African Sharptooth Catfish (*Clarias gariepinus*)

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Simple Summary

The African sharptooth catfish (*Clarias gariepinus*) is widely cultured in aquaculture due to its rapid growth and adaptability to intensive rearing conditions. Understanding the normal structure of its heart is important for comparative morphological studies and for future investigations of cardiovascular adaptations. Although the general anatomy of the heart in this species has been described previously, information on its tissue organization and cellular characteristics remains limited. This study examined the microscopic structure of the atrium and ventricle in ten sexually mature female African sharptooth catfish. The ventricular myocardium consisted predominantly of interconnected muscular bundles forming a well-developed trabecular network, with connective tissue located between the bundles. Muscle and connective tissue components were assessed separately to characterize their relative representation. Ventricular cardiomyocytes varied in width and calculated nuclear volume. The atrial myocardium exhibited a thinner and more loosely organized structure than the ventricular myocardium. In addition to typical cardiomyocytes, elongated spindle-shaped cells and cells with vacuolated sarcoplasm were observed in the atrium. These findings provide additional morphological information on the heart of *C. gariepinus* and may serve as a reference for future studies investigating cardiac structure under different environmental and aquaculture conditions.

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Abstract

The histological organization of the heart of the African sharptooth catfish (*Clarias gariepinus*) remains insufficiently characterized. This study examined the atrial and ventricular myocardium of ten sexually mature, clinically healthy females using hematoxylin-and-eosin-stained sections and morphometric analysis. Results are expressed as mean ± standard error (n = 10 fish). The ventricular myocardium exhibited a predominantly spongy, trabecular architecture with interconnected muscular bundles. Small-, medium-, and large-caliber bundles measured 26.13 ± 3.02, 42.37 ± 1.30, and 63.07 ± 1.70 μm, respectively. Separately assessed estimates of muscle and connective tissue representation were 72.03 ± 1.14% and

28.60 ± 1.55%, respectively; these values were not normalized as complementary fractions of a common myocardial area. Cardiomyocyte widths across three descriptive size categories were 7.63 ± 0.46, 11.27 ± 0.43, and 12.90 ± 0.38 μm; corresponding calculated nuclear volumes were 42.13 ± 1.74, 52.07 ± 2.11, and 66.90 ± 2.72 μm^3. The atrial myocardium was thinner and more loosely organized, with spindle-shaped cells and cells exhibiting sarcoplasmic vacuolization. Their functional identity could not be established using routine histology. These findings provide baseline cardiac morphometric data for future comparative studies.

Keywords: cardiomyocytes; trabecular apparatus; muscle bundles; connective tissue; atrium; ventricle; ray-finned fish; aquaculture

1. Introduction

Over recent decades, aquaculture has become an important component of the global food system. Its development has been accompanied by the diversification of cultured species, improvements in feeding technologies, and the widespread adoption of intensive production systems [1]. Under these conditions, fish species characterized by rapid growth, efficient feed utilization, and a high capacity to adapt to artificial rearing environments are of particular importance.

One such species is the African sharptooth catfish ((*Clarias gariepinus* (Burchell, 1822)). It is cultured in ponds, tanks, recirculating aquaculture systems, and aquaponic facilities. This species exhibits rapid growth and can be reared at high stocking densities [2–4]. However, intensive aquaculture exposes fish to various husbandry-related factors. Changes in water temperature and quality, high stocking densities, operational noise, harvesting, grading, and transportation may affect the physiological condition and welfare of fish [5,6].

An important characteristic of *C. gariepinus* is its ability to breathe atmospheric air. This is facilitated by a specialized suprabranchial organ, which enables the fish to survive for certain periods in water with reduced dissolved oxygen concentrations. Nevertheless, this ability does not eliminate the effects of hypoxia on the organism. Under oxygen-deficient conditions, the frequencies of gill ventilation and air breathing, as well as heart rate, may change [7]. Respiratory behavior is also influenced by water temperature and carbon dioxide concentration [8]. Thus, the ability of the African sharptooth catfish to cope with changing environmental conditions is closely associated with the functioning of its cardiovascular system.

The fish heart maintains blood circulation through the gills and subsequently delivers blood to organs and tissues. Its chambers and associated structures are arranged in series, ensuring unidirectional blood flow. The ventricle performs the principal pressure-generating function: during contraction, it generates the pressure required to propel blood through the gill circulation. The cardiac outflow tract dampens pressure fluctuations and promotes a more uniform flow of blood into the ventral aorta [9].

Ventricular morphology varies considerably among fish species. The ventricle may exhibit a sac-like, tubular, or pyramidal shape, while the relative development of compact and trabecular myocardial components differs among species [10,11]. The trabecular component consists of branching muscular trabeculae separated by endocardium-lined spaces. In contrast, the compact component is composed of more densely arranged cardiomyocytes. The boundary between these components is not always clearly defined; consequently, ventricular myocardial organization can be considered a morphological continuum ranging from predominantly trabecular myocardium to a well-developed compact myocardial layer [10].

Cardiac function depends not only on the relative proportions of compact and trabecular myocardium but also on the orientation of muscle fibers, the thickness of the bundles they form, the amount of connective tissue, and the pattern of blood supply. Connective tissue provides structural support to the myocardium, integrates muscular bundles, and contributes to force transmission during contraction. The arrangement of muscle fibers and connective tissue layers varies among fish species and between different regions of the same ventricle [11,12]. The compact and trabecular components also differ in the organization of intercellular junctions between cardiomyocytes, which are essential for coordinated myocardial contraction [13].

Myocardial structure is associated, to some extent, with the lifestyle and locomotor activity of fish. In more active species, the compact myocardial component and coronary circulation are generally better developed. However, this pattern is not universal. Even among closely related species, ventricular shape, the proportion of compact myocardium, and the distribution of blood vessels may differ substantially [10,14]. Therefore, the structural characteristics of the heart should be investigated separately for individual fish species.

Previous studies of *C. gariepinus* have described a sac-like ventricle with a mixed myocardial organization. Its outer compact component is relatively thin, whereas the inner trabecular component is well developed. Connective tissue layers and blood vessels have also been identified within the myocardium [14,15]. Studies of other freshwater fish have demonstrated that myocardial structure may vary depending on species, age, body mass, and environmental conditions [16,17].

Earlier morphological investigations of the heart of the African sharptooth catfish (*Clarias gariepinus*) have characterized the myocardial architecture of the ventricle, the presence of outer compact and inner spongy myocardial components, and the pattern of coronary vascularization [14,15]. However, a detailed morphometric characterization of the myocardium requires not only an understanding of the overall organization of its structural components but also quantitative data on muscular bundles, cardiomyocytes, their nuclei, and tissue composition.

In a previous study, the authors characterized the topographical, anatomical, and organometric features of the heart of *C. gariepinus* [18]. The present investigation extends this work by examining myocardial tissue organization at the cellular and histomorphometric levels.

The scientific contribution of this study lies in providing a comprehensive set of quantitative characteristics of the ventricular myocardium of *C. gariepinus*, including the width of muscular bundles of different calibers, morphometric parameters of cardiomyocytes and their nuclei, and the relative areas occupied by muscular and connective tissue components. Combining these measurements with the histological characterization of the atrium and ventricle extends existing knowledge of cardiac structural organization in this species reared in a recirculating aquaculture system.

The aim of this study was to characterize the histological organization of the atrium and ventricle of the African sharptooth catfish (*Clarias gariepinus*), determine the morphometric parameters of ventricular muscular bundles and cardiomyocytes, assess the relative areas occupied by muscular and connective tissue components of the myocardium, and identify features of its cellular organization.

The working hypothesis was that the ventricular myocardium of *C. gariepinus* exhibits morphometric heterogeneity in muscular bundles and cardiomyocytes, reflected in differences in their dimensions and accompanied by variation in the morphometric parameters of cardiomyocyte nuclei. It was hypothesized that quantitative characterization of these structures, combined with histological assessment of the compact and spongy myocardial

components, would provide a more detailed understanding of myocardial tissue organization in this species.

2. Materials and Methods

2.1. Study Conditions and Ethical Considerations

The study was conducted at the Department of Zoology, Biological Monitoring and Nature Conservation, Faculty of Natural Sciences, Zhytomyr Ivan Franko State University, as part of the research project "Animals of Artificial and Natural Ecosystems of Ukraine" (state registration number 0122U002270).

All procedures involving experimental animals were carried out in accordance with the principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the Regulations for Conducting Research Involving Experimental Animals approved by Order No. 281 of the Ministry of Health of Ukraine dated 1 November 2000, "On Measures for Further Improvement of the Organizational Framework for Research Involving Experimental Animals," and the Law of Ukraine "On the Protection of Animals from Cruelty" (No. 3447-IV of 21 February 2006, Kyiv). The study also took into account the general principles of Good Laboratory Practice (GLP, 1981) and the "General Ethical Principles of Animal Experimentation," adopted at the First National Congress on Bioethics (Kyiv, 2001).

The study included ten clinically healthy, sexually mature female African sharptooth catfish (*Clarias gariepinus* (Burchell, 1822)), approximately 12 months of age, with a mean body weight of 1271 ± 137 g (mean ± SD) and a total body length ranging from 50 to 55 cm.

The fish were purchased as partially grown individuals and subsequently maintained in a privately operated indoor recirculating aquaculture system (RAS) comprising tanks with a capacity of up to 500 L at a stocking density of 100–120 kg/m^3. The system was equipped with Eheim Professional 3 1200XL filtration units, Resun UV-08 ultraviolet sterilizers (24 W), and Resun ACO-001 air compressors. Water temperature was maintained at 25–28 °C using JBL ProTemp e500 external heaters (500 W). The water supply consisted of previously settled tap water adjusted to the tank temperature, with approximately 10% of the total water volume renewed daily.

Hydrochemical monitoring was performed once daily and included measurements of water temperature, pH, ammonia/ammonium (NH_3/NH_{4^+}), nitrite (NO_{2^-}), and nitrate (NO_{3^-}). According to the routine monitoring results, the concentrations of the monitored nitrogen compounds remained within the acceptable ranges for the culture of *Clarias gariepinus*. Water temperature was measured using an electronic thermometer, pH was determined using an SX-620 laboratory pH meter, and nitrogen compounds were monitored using Ptero water quality test kits. The recorded pH values ranged from 6.5 to 8.5, and dissolved oxygen concentrations were reported to range from 5 to 7 mg/L. The fish were fed compound feed twice daily, in the morning and evening.

The sex of the examined fish was Initially determined based on external sexual characteristics, particularly the morphology of the genital papilla, and subsequently confirmed by macroscopic examination of the gonads during dissection. Sexual maturity was assessed based on ovarian development, considering the size and macroscopic appearance of the ovaries and the presence of visible developing oocytes.

The study was conducted using an available sample of ten sexually mature female fish. The inclusion of females reflected the availability of biological material rather than a predefined selection criterion based on sex. The study was designed to characterize the histological organization and morphometric features of the myocardium in the examined individuals and did not aim to investigate sex-related differences.

The clinical condition of the fish was assessed daily based on locomotor activity, behavior, feeding response, and the presence of external signs of pathology. Only individuals showing no clinical signs of disease were included in the study.

2.2. Sample Collection

Prior to sample collection, the fish were anesthetized by immersion in an aqueous solution of metomidate hydrochloride (Hypnodil; Janssen Pharmaceutica B.V., Beerse, Belgium) at a final concentration of 5–10 mg/L. The depth of anesthesia was assessed based on loss of equilibrium, cessation of voluntary movements, and absence of responses to external stimuli. Following induction of anesthesia, the fish were euthanized by decapitation. The hearts were subsequently dissected free from the surrounding tissues, and samples were collected from corresponding regions of the atrium and ventricle for histological examination.

The macroscopic structure and organometric characteristics of the heart of *Clarias gariepinus* were previously investigated by the authors using a separate sample of fish [18]. In the present investigation, external anatomical landmarks were used solely to identify the cardiac chambers and ensure standardized tissue sampling.

2.3. Histological and Morphometric Examination

Conventional methods of tissue fixation, paraffin embedding, and histological section preparation were used for microscopic examination.

Tissue samples were fixed in a chilled 10–12% neutral formalin solution for 42–50 h, followed by washing under running water, dehydration through a graded ethanol series, and paraffin embedding. Paraffin sections were prepared using an MS-2 sliding microtome (Kharkiv Plant Tochmedprylad, Kharkiv, Ukraine), with a section thickness not exceeding 10 µm. The sections were subsequently deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E) for histological examination and morphometric analysis.

Histological slides were examined using a Micros light microscope (Micros, Austria) equipped with a CAM V-200 digital camera (InterMed, Shenzhen, China). The histological organization of the atrium and ventricle was evaluated in sections obtained from corresponding anatomical regions of each heart. Linear morphometric measurements of ventricular muscle bundles, cardiomyocytes, and cardiomyocyte nuclei were performed using a microscope eyepiece measuring scale, following the general histometric approach described by Horalskyi et al. [19]. According to this approach, the widths of muscle bundles and cardiomyocytes were measured perpendicular to their longitudinal axes, whereas nuclear length and maximum width were determined along the corresponding major and minor axes of the nuclear profiles. For consistency among fish, histological assessment and morphometric measurements were conducted in corresponding anatomically identified ventricular regions. The width of a muscle bundle referred to the transverse dimension of a recognizable multicellular bundle of cardiac muscle fibers, whereas cardiomyocyte width referred to the transverse dimension of an individual cardiomyocyte profile. These measurements characterize different structural levels of the myocardium and were recorded separately. The eyepiece micrometer was calibrated using a stage micrometer to determine the actual length represented by each scale division at the magnifications used for morphometric measurements. Morphometric analysis of the ventricular myocardium included measurements of muscle bundle width, cardiomyocyte width, and nuclear length and width, as well as estimation of the relative areas occupied by muscle and connective tissue.

The relative representation of mus”ular’and connective tissue components was assessed separately in different microscopic fields using an ocular square grid comprising 64 equal squares. Grid intersection points corresponding to the respective tissue components were counted, and the relative area of each component was calculated independently. Because

muscle and connective tissue were evaluated in different microscopic fields, the resulting percentages were not treated as complementary fractions of the same reference area and were not normalized to sum to 100%.

The nuclear volume of cardiomyocytes was estimated using the ellipsoid-based formula previously applied by Horalskyi et al. [19]. Nuclear length (A) and maximum width (B) were measured, and the calculated nuclear volume was determined using the following equation:

$$V = \frac{\pi}{6} \times A \times B^2$$

where V is the calculated nuclear volume (μm^3), A is the nuclear length (μm), B is the maximum nuclear width (μm), and π is approximately 3.14. The resulting values were interpreted as estimates of nuclear volume derived from linear morphometric measurements.

For descriptive morphometric analysis, ventricular muscle bundles and cardiomyocytes were grouped into three size categories (small, medium, and large) according to their observed widths. The categories were assigned descriptively, without predefined numerical cut-off values, and were used to characterize variation in the dimensions of the examined structures. They should not be interpreted as distinct anatomical or functional types.

The terminology used to describe cardiac structures followed international veterinary anatomical and histological nomenclatures.

2.4. Statistical Analysis

For each morphometric parameter, three measurements were obtained from each of the ten examined fish. Individual fish were considered independent biological units ($n = 10$), whereas repeated measurements were treated as technical replicates. For each fish, the arithmetic mean of the three measurements was calculated. Based on the ten individual means, the overall arithmetic mean (Mean), standard deviation (SD), standard error of the mean (SE = SD/$\sqrt{n}$), coefficient of variation (CV = SD/Mean × 100%), and 95% confidence interval (CI) of the mean were calculated using Student's t-distribution.

An exploratory correlation analysis was performed to assess the associations between cardiomyocyte width and calculated nuclear volume using fish-level mean values ($n = 10$). Spearman's rank correlation coefficient (ρ) was calculated separately for the three descriptive cardiomyocyte size categories. The Holm procedure was applied to adjust P-values for multiple comparisons. These analyses assessed associations between fish-level morphometric summaries rather than directly paired measurements from individual cardiomyocytes.

The raw morphometric measurements, individual fish-level means, and summary statistics for all ten fish are provided in Supplementary Table S1.

3. Results

3.1. Microscopic Structure of the Ventricle

The ventricular wall consisted of three layers: the epicardium, myocardium, and endocardium (Figure 1a–c). Numerous elongated intertrabecular spaces extended from the central ventricular cavity into the myocardium, forming an irregular relief of its inner surface.

The epicardium appeared as a thin connective tissue layer covered by mesothelium. Its loose fibrous connective tissue contained blood vessels supplying the cardiac muscle, isolated adipocytes, and local accumulations of adipose tissue (Figure 1b–d).

The myocardium constituted the thickest layer of the ventricular wall and consisted of two structural components: an outer compact layer and an inner spongy (trabecular) layer. Blood vessels, including subepicardial and intramyocardial vascular structures, were observed among the muscle cells of the outer compact component (Figure 2). Their presence indicates that coronary blood supply contributes to myocardial perfusion. However, the

histological findings do not allow direct conclusions to be drawn regarding the functional significance of the observed vascularization under hypoxic conditions.

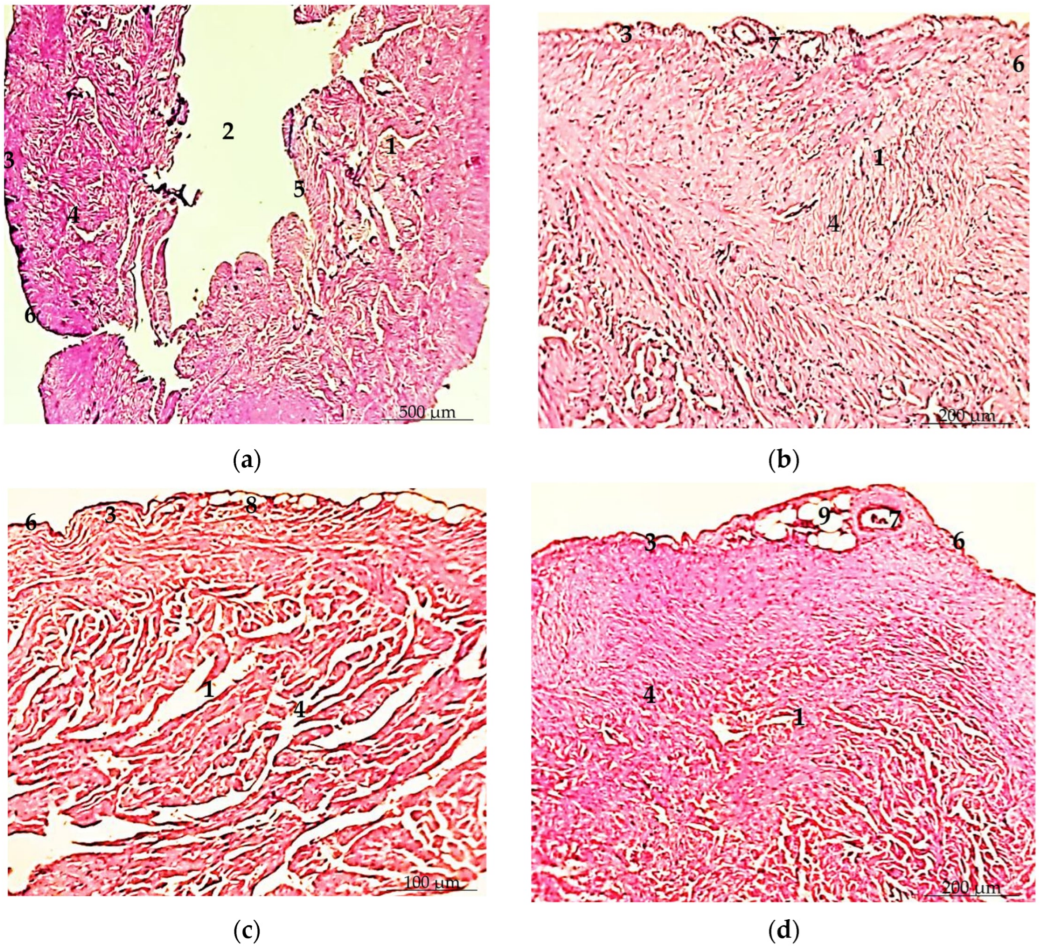
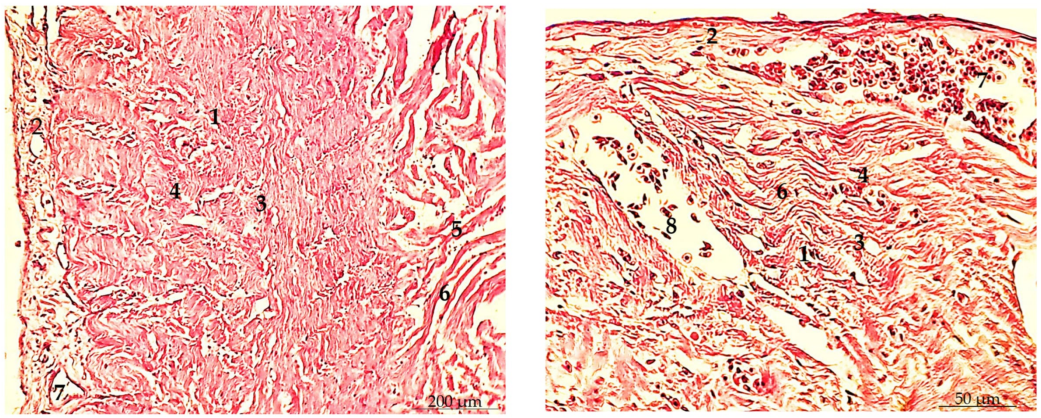


Figure 1. Microscopic structure of the ventricular wall of the African sharptooth catfish (*Clarias gariepinus*): (**a**) ×20; (**b**) ×56; (**c**) ×80; (**d**) ×56. 1—ventricular wall; 2—ventricular cavity; 3—epicardium; 4—myocardium; 5—endocardium; 6—mesothelial membrane; 7—blood vessels; 8—fat cells (adipocytes); 9—aggregation of fat cells. Hematoxylin and eosin (H&E) staining.

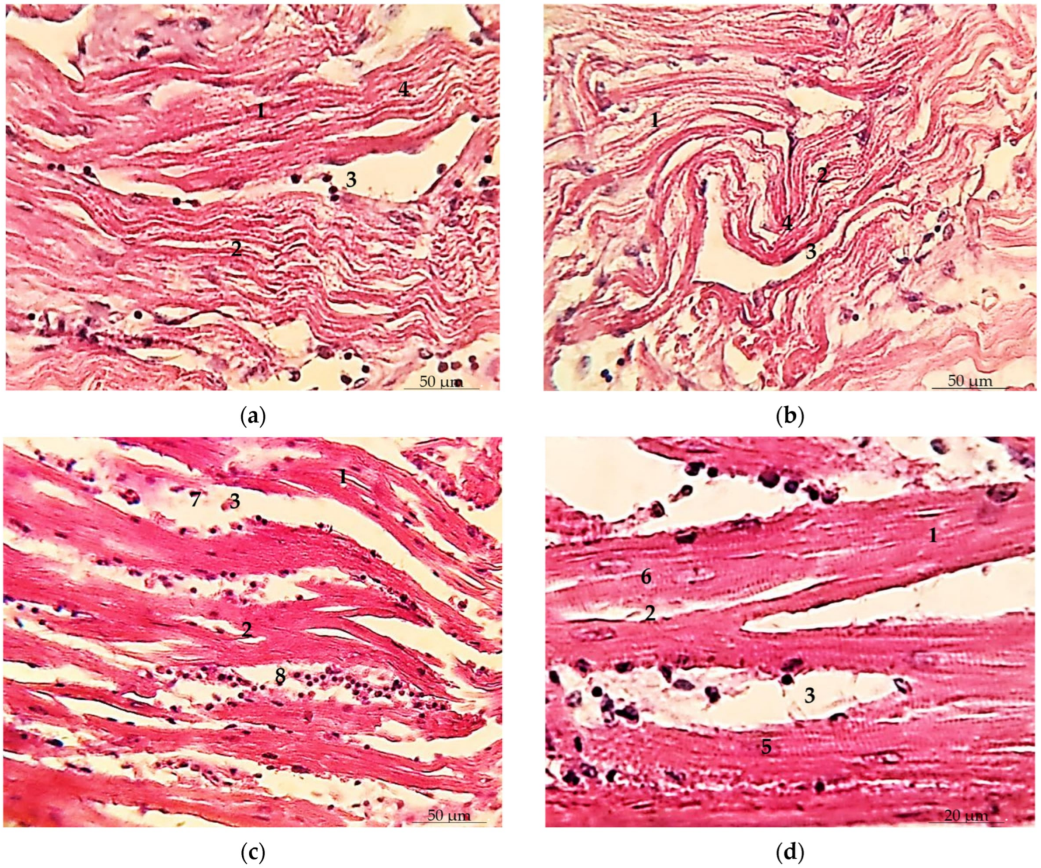
The inner spongy layer was more developed and exhibited a pronounced spongy, or trabecular, architecture. It consisted of bundles of striated cardiac muscle fibers of varying thickness and spatial orientation. The muscular bundles anastomosed with one another, forming a reticular network with numerous intertrabecular spaces (Figure 3a–d). Blood vessels of the microcirculatory network were not clearly visualized in the examined histological sections of the spongy myocardium. This observation reflects the morphological characteristics of the examined myocardial regions but does not allow the complete absence of blood vessels in the spongy component of the ventricle to be established.

The widths of small-, medium-, and large-caliber muscular bundles were 26.13 ± 3.02 µm, 42.37 ± 1.30 µm, and 63.07 ± 1.70 µm, respectively. The separately assessed relative representation of muscle tissue in the ventricular myocardium averaged 72.03 ± 1.14%, whereas the corresponding estimate for connective tissue was 28.60 ± 1.55% (Table 1).



(**a**) (**b**)

Figure 2. Microscopic structure of the ventricle of the African sharptooth catfish (*Clarias gariepinus*) heart: (**a**) ×56; (**b**) ×280. 1—ventricular wall; 2—epicardium; 3—myocardium; 4—outer compact myocardial layer; 5—inner spongy (trabecular) myocardial layer; 6—muscle fibers; 7—blood vessels of the epicardium; 8—blood vessels of the outer compact myocardial layer. Hematoxylin and eosin (H&E) staining.



(**a**) (**b**)

(**c**) (**d**)

Figure 3. Microscopic structure of the ventricular myocardium of the African sharptooth catfish (*Clarias gariepinus*): (**a**) ×240; (**b**) ×240; (**c**) ×240; (**d**) ×400. 1—muscle fibers; 2—bundles of muscle fibers; 3—intermuscular connective tissue; 4—longitudinally oriented fibrillar pattern; 5—transverse striations; 6—cardiomyocyte nuclei; 7—individual blood cells; 8—localized aggregations of blood cells. Hematoxylin and eosin (H&E) staining.

Table 1. Histomorphometric characteristics of the ventricular myocardium in the African sharptooth catfish (*Clarias gariepinus*).

Parameters	Mean ± SE	95% CI	SD	CV, %
Small-caliber muscle bundles (μm)	26.13 ± 3.02	19.30–32.96	9.54	36.52
Medium-caliber muscle bundles (μm)	42.37 ± 1.30	39.43–45.31	4.12	9.72
Large-caliber muscle bundles (μm)	63.07 ± 1.70	59.22–66.92	5.37	8.51
Separately estimated relative area of muscle tissue (%)	72.03 ± 1.14	69.45–74.61	3.60	5.00
Separately estimated relative area of connective tissue (%)	28.60 ± 1.55	25.09–32.11	4.90	17.12

Note: Mean—arithmetic mean; SE—standard error of the mean; 95% CI—95% confidence interval of the mean; SD—standard deviation; CV—coefficient of variation (%); $n = 10$ fish. The relative areas of muscle and connective tissue were assessed and recorded separately. The reported estimates were not subsequently normalized to sum to 100% and should not be interpreted as a verified complementary partition of the same reference area.

Cardiomyocytes were elongated and exhibited a longitudinally oriented fibrillar pattern, whereas transverse striations were less clearly discernible in the H&E-stained sections. The cells were connected at their ends by intercalated discs. The nuclei were predominantly located in the central region of the cells, although eccentric positioning was occasionally observed. They were oval or rod-shaped and contained one or occasionally two nucleoli.

The widths of small-, medium-, and large-sized cardiomyocytes were 7.63 ± 0.46, 11.27 ± 0.43, and 12.90 ± 0.38 μm, respectively. Their calculated nuclear volumes were 42.13 ± 1.74, 52.07 ± 2.11, and 66.90 ± 2.72 μm^3, respectively (Table 2).

Table 2. Histomorphometric characteristics of ventricular cardiomyocytes in the African sharptooth catfish (*Clarias gariepinus*).

Parameters	Mean ± SE	95% CI	SD	CV, %
Width of small-sized cardiomyocytes (μm)	7.63 ± 0.46	6.59–8.67	1.45	19.03
Width of medium-sized cardiomyocytes (μm)	11.27 ± 0.43	10.30–12.24	1.35	11.98
Width of large-sized cardiomyocytes (μm)	12.90 ± 0.38	12.04–13.76	1.21	9.36
Calculated nuclear volume of small-sized cardiomyocytes (μm^3)	42.13 ± 1.74	38.19–46.07	5.49	13.03
Calculated nuclear volume of medium-sized cardiomyocytes (μm^3)	52.07 ± 2.11	47.30–56.84	6.68	12.83
Calculated nuclear volume of large-sized cardiomyocytes (μm^3)	66.90 ± 2.72	60.75–73.05	8.62	12.88

Note: Data are presented as the arithmetic mean ± standard error of the mean (mean ± SE), 95% confidence interval of the mean (95% CI), standard deviation (SD), and coefficient of variation (CV, %); $n = 10$ independent fish. The cardiomyocyte size categories were used for descriptive morphometric purposes only.

Exploratory Spearman correlation analysis revealed a positive association between mean cardiomyocyte width and calculated nuclear volume in the small-sized category ($\rho = 0.649$, $p = 0.042$). However, this association did not remain statistically significant after Holm's correction for multiple comparisons (adjusted $p = 0.127$). No statistically significant associations were detected in the medium-sized ($\rho = -0.335$, adjusted $p = 0.687$) or large-sized ($\rho = 0.189$, adjusted $p = 0.687$) categories. These results should be interpreted cautiously because the analysis was based on fish-level mean values rather than matched measurements from individual cells.

Layers of loose fibrous connective tissue were present between the bundles of muscle fibers. These layers contained scattered fibrocytes, blood cells, and localized accumulations of blood cells. Blood vessels of the microcirculatory network were not clearly visualized in the examined sections of the spongy myocardium.

The endocardium consisted of a single layer of flattened endothelial cells and a thin subendothelial layer of loose fibrous connective tissue. It lined the inner surface of the ventricle and covered its trabeculae.

3.2. Microscopic Structure of the Atrium

The atrial wall also consisted of three layers: the epicardium, myocardium, and endocardium (Figures 4 and 5). However, its structure differed from that of the ventricular wall primarily in its reduced thickness and the looser arrangement of muscular elements.

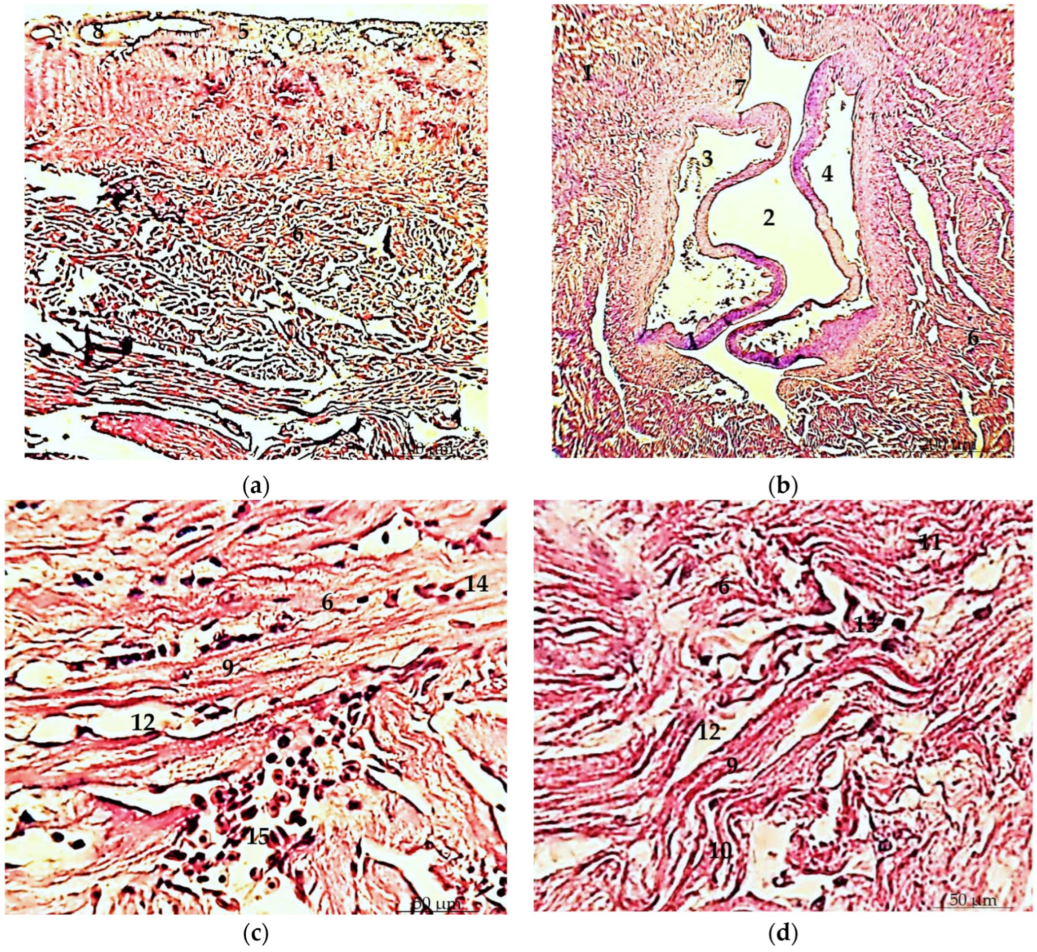


Figure 4. Microscopic structure of the atrium of the African sharptooth catfish (*Clarias gariepinus*) heart: (**a**) ×80; (**b**) ×56; (**c**) ×240; (**d**) ×240. 1—atrial wall; 2—atrial cavity; 3 and 4—regions of the atrial cavity partially separated by an incomplete muscular ridge; 5—epicardium; 6—myocardium; 7—endocardium; 8—blood vessels; 9—muscle fibers; 10—longitudinally oriented fibrillar pattern; 11—transverse striations; 12—intermuscular connective tissue; 13—fibrocytes; 14—individual blood cells; 15—aggregations of blood cells. Hematoxylin and eosin (H&E) staining.

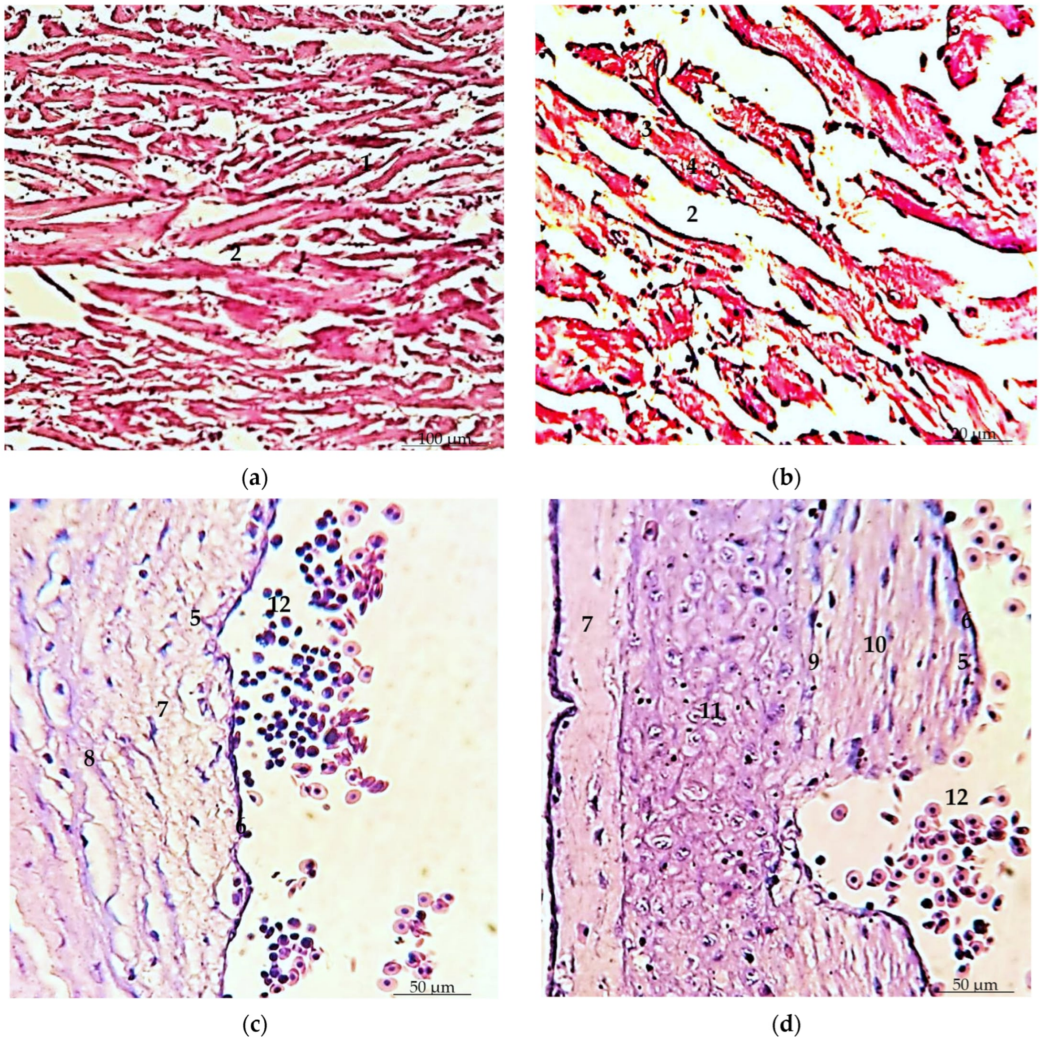


Figure 5. Microscopic structure of the atrium of the African sharptooth catfish (*Clarias gariepinus*) heart: (**a**) ×80; (**b**) ×400; (**c**) ×240; (**d**) ×240. 1—elongated spindle-shaped cells; 2—intermuscular connective tissue; 3—cells with vacuolated sarcoplasm; 4—nuclei of cells with vacuolated sarcoplasm; 5—endocardium; 6—endothelial layer; 7—loose fibrous connective tissue of the endocardium; 8—atrial wall region adjacent to the lumen; 9—muscular region bordering the incomplete atrial ridge; 10—elongated muscular profiles; 11—clustered cellular profiles within the ridge; 12—accumulations of blood cells. Hematoxylin and eosin (H&E) staining.

The atrial epicardium consisted of loose fibrous connective tissue containing blood vessels and connective tissue fibers oriented in various directions. Its outer surface was covered by mesothelium.

The atrial myocardium consisted of thin bundles of cardiomyocytes that anastomosed with one another, forming a loose reticular network with large intertrabecular spaces. Scattered blood cells and their accumulations were observed within the intermuscular connective tissue, predominantly in the subepicardial regions. However, coronary vessels were not identified in these regions.

Typical cardiomyocytes were elongated and exhibited a longitudinally oriented fibrillar pattern, whereas transverse striations were less clearly discernible in the H&E-stained sections. The nuclei were predominantly centrally located, oval or rod-shaped, and contained one, occasionally two, nucleoli.

In addition to typical contractile cardiomyocytes, individual elongated, spindle-shaped cells were observed in the atrial myocardium (Figure 5a). Cells exhibiting areas of sar-

coplasmic clearing and vacuolization were also identified (Figure 5b). These morphological features indicate structural heterogeneity within the cellular composition of the atrial myocardium. However, they do not provide sufficient evidence to definitively establish the conductive or secretory specialization of the observed cells.

The atrial endocardium consisted of an endothelial layer, a basement membrane, and a subendothelial layer of loose fibrous connective tissue. An incomplete muscular ridge was observed within the atrial cavity, partially separating it into two regions. Blood cells were observed near the endocardial surfaces and within the atrial lumen (Figure 5c,d).

4. Discussion

The present study demonstrated that the heart of the African sharptooth catfish (*Clarias gariepinus*) generally retains the typical structural organization of teleost fish while exhibiting distinct species-specific features. The sinus venosus, atrium, ventricle, and bulbus arteriosus form a continuous pathway for blood flow. A similar cardiac organization is characteristic of other ray-finned fishes, although the shape, size, and relative proportions of individual cardiac components may vary considerably depending on lifestyle, locomotor activity, and respiratory adaptations [20,21].

The structural organization of the ventricular and atrial myocardium reflects differences in the functional demands placed on these cardiac chambers. The ventricle pumps blood into the branchial vascular system, whereas the atrium primarily receives blood and transfers it to the ventricle. The observed differences in histoarchitecture are consistent with established structural and functional characteristics of the vertebrate heart [22].

A particularly prominent feature of the ventricle was its spongy, or trabecular, architecture. Muscular bundles of varying thickness interlaced and anastomosed, forming a three-dimensional network with numerous intertrabecular spaces. This organization increases the contact area between blood and the myocardium and reduces the diffusion distance for oxygen and nutrients to reach cardiomyocytes. Trabeculation is an evolutionarily ancient feature of the vertebrate heart, although the number, thickness, and arrangement of trabeculae vary considerably among taxonomic groups [23]. In teleost fish, the trabecular myocardium may constitute almost the entire thickness of the ventricular wall or coexist with an outer compact layer.

A thin outer compact layer and a well-developed inner spongy myocardium have previously been described in *Clarias gariepinus* [15]. In the histological sections examined in the present study, the spongy component predominated, whereas the compact layer was less prominent. However, the available morphometric data did not allow a quantitative assessment of its relative thickness.

Blood vessels of the microcirculatory network were not clearly identified in the examined regions of the spongy myocardium. In contrast, vessels within the compact myocardium of *C. gariepinus* have been documented in previous morphological studies [15]. These observations are consistent with the hypothesis that the spongy component receives oxygen primarily from blood within the intertrabecular spaces, whereas oxygen delivery to the compact component involves the coronary circulation. A more detailed assessment of myocardial vascularization would require serial histological sections and specialized vascular visualization techniques.

Thus, unlike many other relatively sedentary fish species, in which myocardial oxygen supply depends predominantly on diffusion from blood within the ventricular lumen and intertrabecular spaces, *Clarias gariepinus* exhibits a combination of luminal and coronary pathways of myocardial oxygen delivery. The presence of a coronary vascular system is associated with the two-layered organization of the ventricular wall, comprising an outer compact myocardium and an inner spongy myocardium [14]. According to Simões

et al. [15], the coronary artery originates from the fourth branchial arch, extends along the dorsal surface of the bulbus arteriosus, and branches over the ventral and dorsal surfaces of the ventricle. Its subepicardial branches form a vascular network supplying the compact myocardial layer, whereas the spongy layer receives oxygen predominantly from venous blood entering the ventricular cavity.

The two-component organization of the ventricular myocardium in *C. gariepinus* may have functional significance. The compact layer may contribute to the mechanical efficiency of ventricular contraction, whereas the trabecular network provides an extensive contact area between cardiomyocytes and blood within the ventricular cavity [24,25]. In the single circulatory system of fish, the ventricle generates the pressure required to drive blood through the branchial vascular bed and subsequently into the systemic circulation [26]. However, direct hemodynamic measurements were not performed in the present study.

The atrial myocardium differed from the ventricular myocardium in having thinner muscular bundles and a more loosely arranged reticular architecture. Coronary vessels were not identified in the examined atrial histological sections; however, this observation does not establish their complete absence throughout the atrium. Luminal oxygen diffusion may play an important role in supplying atrial cardiomyocytes.

The muscular ridge identified within the atrial cavity partially divided the chamber. However, based on histological examination alone, this structure should not be regarded as a fully developed interatrial septum. Establishing its anatomical organization would require a three-dimensional assessment of the heart, such as serial histological reconstruction or micro-computed tomography.

In addition to typical cardiomyocytes, elongated spindle-shaped cells and cells exhibiting sarcoplasmic vacuolization were observed in the atrial myocardium. Their functional specialization requires further confirmation. The presence of these cellular forms indicates morphological heterogeneity within the examined myocardial tissue [27,28]. Nevertheless, light microscopic examination of hematoxylin-and-eosin-stained sections does not allow their conductive or secretory specialization to be established. Further investigations, particularly immunohistochemical and ultrastructural analyses, are required to determine the functional identity of these cells.

The ability of the African sharptooth catfish to breathe atmospheric air undoubtedly influences its cardiovascular physiology. However, *C. gariepinus* is not a lungfish and does not possess true lungs. Air breathing is facilitated by paired suprabranchial chambers containing arborescent respiratory organs. Studies of other air-breathing catfish have demonstrated that blood oxygenated in accessory respiratory organs returns to the general circulation and partially mixes with venous blood [21]. Therefore, the muscular ridge observed in the atrium cannot automatically be interpreted as evidence of a separate pulmonary circulation.

The present findings contribute to the comparative morphological understanding of the hearts of air-breathing teleost fish. Of particular value are the quantitative characteristics of ventricular muscular bundles, tissue components, and cardiomyocytes.

The observed morphometric heterogeneity of ventricular muscular bundles and cardiomyocytes in *Clarias gariepinus* indicates structural diversity within its myocardium. The findings characterize the dimensional features of cellular and tissue components but do not allow their functional specialization to be directly established. Given the air-breathing ability of this species, future investigations should focus on the potential relationships between quantitative myocardial characteristics and oxygen availability, particularly through comparisons of fish maintained under different water oxygenation regimes.

Comparative morphological studies have demonstrated that the relative proportions of compact and spongy myocardium, as well as the degree of coronary vascular development, vary considerably among fish species. In *C. gariepinus*, a two-layered myocardium and

subepicardial arterial branches contributing to vascularization of the compact layer have previously been described [29]. In a comparative study, Tota et al. demonstrated that different combinations of arterial and luminal blood supply may be associated with distinct patterns of ventricular organization [30]. Similarly, a predominance of the trabecular component, accompanied by coronary vessels within the compact myocardium, has been reported in three Neotropical teleost species [31]. These findings emphasize the need for caution when interpreting the present morphological observations in an interspecific comparative context.

Study Limitations

The study included a relatively small number of clinically healthy fish ($n = 10$), which limits the assessment of individual variability in morphological parameters and the generalizability of the findings to broader populations of *C. gariepinus*. Sex-related differences could not be assessed because only females were examined; the available data were also insufficient to evaluate age- and body-size-related effects separately. Therefore, the morphometric characteristics obtained should be regarded as preliminary findings specific to the examined sample.

Histological assessment was performed using hematoxylin and eosin (H&E) staining, which allowed characterization of the general myocardial architecture but provided limited differentiation between muscle tissue and collagenous connective tissue. Consequently, the reported estimates of connective tissue representation should be interpreted with caution. Furthermore, H&E staining alone was insufficient to confirm the secretory or conductive specialization of individual cardiomyocytes or to comprehensively characterize the coronary vascular network. Specialized histochemical staining, immunohistochemical labeling, and three-dimensional reconstruction were not performed. Future studies should incorporate collagen-specific staining methods, such as Masson's trichrome or picrosirius red, together with appropriate immunohistochemical techniques and standardized digital image analysis to improve the accuracy of connective tissue quantification and provide a more detailed characterization of myocardial cellular and vascular structures.

The relative representation of muscle and connective tissue was assessed separately, and the recorded estimates did not consistently sum to 100%. Their combined mean value was 100.63%. Therefore, these estimates should be interpreted as separately recorded morphometric parameters rather than as a validated complementary partition of a single myocardial area. The available dataset does not permit retrospective verification of the reference areas used for each measurement. Standardized image segmentation within identical regions of interest, combined with connective tissue-specific staining, would be necessary to establish precise complementary tissue-area fractions.

The reported ranges of formalin concentration (10–12%) and fixation duration (42–50 h), together with the use of histological sections thinner than 10 µm, represent methodological considerations, as the extent of variation in tissue processing among individual samples was not established. Although the section thickness was less than 10 µm, variability in section preparation may have affected the precision of morphometric measurements by complicating the identification of cardiomyocyte boundaries and nuclear profiles. Therefore, the reported cardiomyocyte dimensions and calculated nuclear volumes should be interpreted with caution. Future investigations using standardized fixation conditions, precisely controlled section thickness, and calibrated image analysis procedures are recommended to improve measurement precision and reproducibility.

Three measurements of each morphometric parameter were obtained from each fish, and the results were used to calculate individual mean values. This approach allowed within-individual variability to be taken into account. However, intraobserver and interobserver

measurement agreement was not independently assessed, which should be considered when interpreting the findings. Future investigations should incorporate independent repeated measurements and quantitative assessments of measurement reproducibility.

5. Conclusions

The ventricular myocardium of the African sharptooth catfish (*Clarias gariepinus*) exhibited a predominantly spongy architecture, consisting of multidirectionally oriented bundles of cardiomyocytes that anastomosed to form an extensive branching trabecular network. Muscle tissue constituted the predominant component of the myocardium, whereas connective tissue formed layers between the muscular bundles. Cardiomyocytes exhibited variation in width and calculated nuclear volume across the descriptively defined size categories.

The atrial myocardium exhibited a thinner and more loosely organized reticular architecture than the ventricular myocardium, consistent with the different functional demands placed on these chambers. In addition to typical contractile cardiomyocytes, elongated spindle-shaped cells and cells exhibiting sarcoplasmic vacuolization were observed in the atrial myocardium. These findings indicate morphological heterogeneity within the examined tissue; however, the functional identity of these cells cannot be established on the basis of hematoxylin and eosin staining alone. Further immunohistochemical and ultrastructural investigations are required to clarify their nature.

The histological and morphometric findings provide additional information on the cardiac structure of *C. gariepinus* under the examined conditions and may serve as baseline data for future comparative morphological studies. Their suitability as indicators of adaptive or pathological changes requires further validation.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ani16233785/s1, Table S1: Raw morphometric measurements of cardiac tissue in *Clarias gariepinus*, including three technical replicates per fish, individual fish-level means, and summary statistics ($n = 10$).

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the findings of this study are available in the Supplementary Materials (Supplementary Table S1). Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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